The Enzymatic Synthesis of Thiamine Monophosphate

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The catalysis, by the enzyme thiaminase, of reversible base-exchange reactions between thiamine and aromatic tertiary amines was first reported by Fujita et al. (1). Related findings with mammalian diphosphopyridine nucleotidases (2) and with methionine activation (3) drew attention to the energy-rich nature of "onium" (4-6) compounds and suggested that the alkylation of thiazole must involve activation of the pyrimidine, 2-methyl-4-amino-5-hydroxymethylpyrimidine. That the pyrimidine might be activated by phosphorylation was suggested by the observation of Harris and Yavit (7) that in crude extracts of bakers' yeast, the phosphate ester of the pyrimidine was a better precursor of thiamine than the pyrimidine compound. In independent studies, Leder (8) and Nose, Ueda, and Kawasaki (9), using synthetic substrates, showed that the reactants were the pyrophosphate ester of the pyrimidine and a phosphorylated thiazole and that the initial reaction product was not thiamine, but a phosphorylated form of thiamine subsequently identified (10, 11) as thiamine monophosphate. Caminer and Brown also showed (12) that 2-methyl-4-amino-5-hydroxymethyl pyrimidine monophosphate and a second pyrimidine derivative identified, on presumptive evidence, as the corresponding thiamine phosphate, were formed enzymatically in partially purified yeast preparations from 2-methyl-4-amino-5-hydroxymethyl pyrimidine and adenosine triphosphate. Evidence for the sequential formation of these esters by separate enzymes has been reported by Lewin and Brown (13). The results of these studies may be summarized by the following equations:

\[
\text{Hydroxymethylpyrimidine} + \text{ATP} \rightarrow \text{hydroxymethylpyrimidine-PP + ADP} \tag{1}
\]

\[
\text{Hydroxymethylpyrimidine-PP + ATP} \rightarrow \text{hydroxymethylpyrimidine-PP + ADP} \tag{2}
\]

\[
\text{Thiazole + ATP} \rightarrow \text{thiazole-P + ADP} \tag{3}
\]

\[
\text{Hydroxymethylpyrimidine PP + thiazole P} \rightarrow \text{thiamine-P + PP} \tag{4}
\]

In this paper, the synthesis of the phosphorylated pyrimidines and the purification and properties of the enzyme that catalyzes reaction (4) are described. The name, thiamine-P pyrophosphorylase, is proposed for this enzyme.

The abbreviations used are: hydroxymethylpyrimidine, 2-methyl-4-amin-5-hydroxymethylpyrimidine; thiazole, 4-methyl-5-(β-hydroxyethyl) thiazole: DEAE-cellulose, N,N-diethylaminoethyl cellulose.

EXPERIMENTAL PROCEDURE

Methods

Thiamine and thiamine phosphate were determined fluorimetrically by oxidation to the corresponding thiochromes with ferricyanide (14) or cyanogen bromide (15), the fluorescence generally being measured directly without extraction into n-hexanol or isobutanol. The CNBr procedure was modified as follows. A sample containing 0.01 to 0.2 mpmole of thiamine in 0.5 ml was acidified by the addition of 0.025 ml of 2.5 N HCl. In enzymatic studies, this stopped the reaction. This was followed in turn, with mixing after each addition, by 0.3 ml of 0.3 ml of CNBr (prepared by decolorizing ice-cold saturated bromine water with 10% KCN) and 0.2 ml of 30% sodium hydroxide. The fluorescence was measured in a Farrand photofluorometer. Stable levels of fluorescence are not obtained by the CNBr procedure if samples are acidified with trichloroacetic acid in place of hydrochloric acid.

Thiamine-P could be distinguished from thiamine by extracting the thiochrome reaction mixture with n-hexanol. Under these conditions, thiochrome, but not thiochrome-P, is extracted. Phosphatase was determined by the method of Fiske and Subbarow (16) and by the sensitive method of Chen (17). For total phosphate, ashing was carried out with magnesium nitrate as described by Ames and Dubin (18).

Materials

Thiamine-P and protamine sulfate were obtained from Nutritional Biochemicals Corporation and thiamine-PP from Schwarz BioResearch, Inc. 2-Methyl-4-amino-5-bromomethylpyrimidine dihydrobromide and 4-methyl-5-(β-hydroxyethyl) thiazole were kindly supplied by Merck and Company, Inc. Thiazole-P and thiazole-PP were obtained by bisulfite cleavage of the corresponding thiamine esters (19).

Hydroxymethylpyrimidine was prepared from the corresponding bromide dihydrobromide by treating an aqueous solution with 3 equivalents of silver nitrate. The silver bromide was removed by filtration, washed with water, and the combined filtrates were made alkaline with sodium hydroxide. The hydroxymethylpyrimidine was obtained in 80 to 90% yield by evaporating to a small volume under reduced pressure. The compound has a marked positive temperature solubility coefficient and is readily recrystallized from water.

Hydroxymethylpyrimidine-P and hydroxymethylpyrimidine-PP were prepared as follows: Hydroxymethylpyrimidine dihydrochloride, 1 g, was added in several small portions to 8 g of pyro-
phosphoric acid at approximately 70°, and the mixture stirred at 105-110° for 20 minutes. The syrup was poured into 100 ml of rapidly stirred ethanol at −15° followed by the addition of 300 ml of ether. The flocculent precipitate was collected by centrifugation, washed twice with ether and dried over silica gel under reduced pressure. The white hygroscopic solid, weighing approximately 1.5 g, was dissolved in cold water, adjusted to pH 6.5, and poured on to a column (2 x 18 cm) of Dowex 50-X8 H⁺, and the pyrimidine compounds were eluted with water. Inorganic phosphate and hydroxymethylpyrimidine-PP appeared immediately after the hold-up volume. Hydroxymethylpyrimidine-P was eluted after approximately 8 bed-volumes of water had run through the column and was obtained as a white solid weighing 300 mg after evaporating the eluate (600 ml) to a small volume under reduced pressure. Hydroxymethylpyrimidine-P was also prepared by the method of Makino and Koike (20).

The hydroxymethylpyrimidine-P solution, adjusted to pH 7.0, was adsorbed on a 3- x 10-cm column of Dowex 1-formate and recovered by gradient elution with ammonium formate with a mixing volume of 500 ml and approaching 1 M ammonium formate. Elution was followed by observing ultraviolet quenching by spots applied to paper. After most of the ammonium formate was removed by lyophilization, the hydroxymethylpyrimidine-PP was isolated as the barium salt, weighing 340 mg.

Properties of Pyrimidine Phosphates—The ultraviolet absorption spectra of hydroxymethylpyrimidine-P and hydroxymethylpyrimidine-PP were identical with that of hydroxymethylpyrimidine, each having a single maximum at 246 nm in 0.1 N HCl. Preparations of both compounds were free of orthophosphate and, on the basis of the molar extinction coefficient of approximately 11.2 x 10⁴ cm² per mole, contained the requisite molar ratio of organic phosphate (Table I). It is of particular interest that the phosphate groups of both pyrimidines are readily hydrolyzed by acid. Fig. 1 shows that the rate of hydrolysis of the two phosphate groups of hydroxymethylpyrimidine-PP by 1 N H₂SO₄ at 100° is as rapid as that of the acid-labile phosphate groups of ATP. Hydroxymethylpyrimidine-P is hydrolyzed at a slower rate, but is completely hydrolyzed by 2 N sulfuric or hydrochloric acid at 100° in approximately 20 minutes. Hydrolysis is somewhat slower in 1 N acid; 30 and 77% in 10 and 20 minutes for hydroxymethylpyrimidine-P and 60 and 90% for hydroxymethylpyrimidine-PP under the same conditions. These results are somewhat at variance with the report of Camiener and Brown (11), who characterized hydroxymethylpyrimidine-P as acid-stable and hydroxymethylpyrimidine-PP acid-labile with respect to hydrolysis to hydroxymethylpyrimidine by 1 N HCl. Under relatively mild conditions, 1 N HCl, 37°, the pyrophosphate and phosphate esters are slowly hydrolyzed to hydroxymethylpyrimidine.

**Purification of Enzyme**

**Assay**—The incubation mixture of 0.5 ml contained the following in micromoles: glycine buffer, pH 9.2, 50; MgSO₄, 0.25; MgCl₂, 0.05; mercaptoethanol, 0.5; thiazole-P, 1.0; and hydroxymethylpyrimidine-PP, 1.0. The reaction was initiated by addition of the enzyme suitably diluted in 0.005 M mercaptoethanol. After 5 minutes at 37°, the reaction was stopped by the addition of 0.5 ml of 10% trichloroacetic acid, and the thiamine phosphate formed was assayed by the ferricyanide thiocarbamide procedure. To a 0.1 ml aliquot was added 0.2 ml of 4 M potassium acetate, followed by 0.1 ml of 0.0036 M potassium ferriyanide in 7 N sodium hydroxide and, after mixing, by 0.1 ml of 0.06% hydrogen peroxide in 5.5 M sodium dihydrogen phosphate, and 0.5 ml of water. Thiamine phosphate formation, measured by this procedure or by the cyanogen bromide reaction described under "Methods," was proportional to enzyme concentration. A unit of activity is defined as that amount of enzyme catalyzing the synthesis of one micromole of thiamine-P at 37° in 5 minutes. Protein was determined by the method of Sutherland et al. (21) or of Warburg and Christian (22).

![Fig. 1. Acid hydrolysis of ATP (O—<>) and hydroxymethylpyrimidine-PP (—<>).](http://www.jbc.org/)<br>

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**Table I**

Properties of pyrimidine compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>Molar ratio of phosphate</th>
<th>Solvent system</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Hydrolyzable</td>
</tr>
<tr>
<td>Hydroxymethylpyrimidine</td>
<td>0.00</td>
<td>0.04</td>
</tr>
<tr>
<td>Hydroxymethylpyrimidine-PP</td>
<td>2.0</td>
<td>2.1</td>
</tr>
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</table>

The molar ratio of phosphate was determined on the basis of a molar extinction coefficient of 1.2 × 10⁴ cm² per mole. Hydrolyzable phosphate represents phosphate liberated in 20 minutes by 2 N HCl at 100°. The solvent systems for paper chromatography were: Solvent A, 35% formic acid-acetone (1:1); Solvent B, 50% acetic acid-acetone (1:1); Solvent C, isobutyric acid-1 N NH₃·OH (10:6). Chromatograms were run ascending in Solvents A and B and descending in Solvent C.
Purification—Anheuser-Busch bakers' yeast, 1.7 kilos, which had been frozen in liquid nitrogen, was thawed and allowed to stand for 18 to 24 hours at 3°C. The mixture was centrifuged and the supernatant liquid (crude extract, 650 ml, Table II) was adjusted to pH 6.5 with 5 N ammonium hydroxide, and a slight excess of 2.5% protamine sulfate was added. Approximately 0.05 the volume of the original extract was usually sufficient. The protamine precipitate was discarded, and the supernatant solution was fractionated with solid ammonium sulfate.

The solution was brought to 50% saturation by the addition of 29.1 g of ammonium sulfate per 100 ml, and the precipitate was discarded. The supernatant was adjusted to 60% saturation by the addition of 6 g of ammonium sulfate per 100 ml and the precipitate dissolved in water and dialyzed for 18 to 24 hours at 3°C. The mixture was centrifuged and the supernatant solution was fractionated with solid ammonium sulfate.

The precipitate was discarded and the supernatant fluid (heated extract) adjusted to pH 6.5 with 5 N ammonium hydroxide, and a slight excess of 2.5% protamine sulfate was added. Approximately 0.05 the volume of the original extract was usually sufficient. The protamine precipitate was discarded, and the supernatant solution was fractionated with solid ammonium sulfate.

The solution was brought to 50% saturation by the addition of 29.1 g of ammonium sulfate per 100 ml, and the precipitate was discarded. The supernatant was adjusted to 60% saturation by the addition of 6 g of ammonium sulfate per 100 ml and the precipitate dissolved in water and dialyzed for 18 to 24 hours with efficient internal stirring against several changes of 6 to 7 liters of 0.005 M mercaptoethanol-0.002 M EDTA (ammonium sulfate I). All solutions containing EDTA were prepared from a 0.2 M stock solution adjusted to pH 7.0.

The dialyzed enzyme was treated serially with 1 ml portions of 2.5% protamine sulfate until less than 5% of the activity remained in the supernatant. The precipitation of the enzyme by protamine was dependent upon the low ionic strength of the same buffer. The enzyme generally appeared after the first

### Table II

<table>
<thead>
<tr>
<th>Step</th>
<th>Volume (ml)</th>
<th>Total activity (mumoles/min)</th>
<th>Specific activity (mumoles/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract</td>
<td>650</td>
<td>101</td>
<td>0.0021</td>
</tr>
<tr>
<td>Heated extract</td>
<td>627</td>
<td>66</td>
<td>0.016</td>
</tr>
<tr>
<td>Ammonium sulfate I</td>
<td>133</td>
<td>39</td>
<td>0.18</td>
</tr>
<tr>
<td>Protamine extract</td>
<td>51</td>
<td>29</td>
<td>0.35</td>
</tr>
<tr>
<td>Ammonium sulfate II</td>
<td>21.5</td>
<td>18</td>
<td>1.1</td>
</tr>
<tr>
<td>DEAE-cellulose</td>
<td>12</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Optima**—Thiamine and its phosphates exhibit very unusual acid-base properties involving a complex series of equilibria with zwitterion, pseudo-base and thiol forms the interrelationship of which has been summarized by Metzler (23). Since the average pK of the diprotonic transition between thiazolium thiamine and the open ring thiol form is approximately 9.3, it is likely that the observed pH optimum of approximately 9.2 (Fig. 2), may reflect acid-base equilibria of thiamine phosphate rather than of charged groups on the active site of the enzyme. **Metal Requirements**—Various preparations were stimulated 3- to 6-fold by 1 x 10^-3 M magnesium. Manganese was approximately half as effective and calcium, zinc, and cobalt were inactive.

**Specificity**—The enzyme is specific for thiazole-P and hydroxymethylpyrimidine-PP. No reaction occurred when thiazole, or thiazole-PP replaced thiazole-P or when hydroxymethylpyrimidine or hydroxymethylpyrimidine-PP replaced hydroxymethylpyrimidine-PP. The same results were obtained in the presence of ATP, indicating that the corresponding kinases are also absent. About half the enzyme preparations of approximately the same specific activity contained no detectable inorganic pyrophosphatase. Other preparations were variously contaminated with pyrophosphatase up to 10% of the thiamine-P pyrophosphorylase activity.

**Stoichiometry**—The results of balance studies, presented in Table III, are in accord with the reaction expressed in Equation 4. In Experiments 1 to 3, hydroxymethylpyrimidine-PP and PP, were assayed as acid-labile phosphate after having been separated by adsorption of the pyrimidine on 10 to 50 mg of Norit A essentially as described for the analysis of nucleotides by Crane (24). The hydroxymethylpyrimidine-PP, adsorbed on charcoal, was hydrolyzed in 1 ml of 2 N HCl at 100°C for 20 minutes. The supernatant was removed, the charcoal was

![Fig. 2. Enzyme activity as a function of pH in glycylglycine (Δ) histidine (O) and glycine (●) buffers.](http://www.jbc.org)
The sensitivity of the CNBr fluorimetric method for thiamine-P was essentially the same as that of a similar solution of hydroxyinhibition by PPi is not competitive with either substrate. In thiazole-P decreased by less than 5% during the 5-minute incubation. Figs. 3 and 4 show the effect of each substrate and of the concentrations of hydroxymethylpyrimidine-PP and of thiamine-P determined fluorimetrically and the other components as described in the text.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Experiment</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroxymethylpyrimidine-PP</td>
<td>µmole</td>
<td>-0.43</td>
<td>-0.30</td>
<td>-0.47</td>
<td>+0.21</td>
</tr>
<tr>
<td>Thiamine-P</td>
<td>µmole</td>
<td>+0.48</td>
<td>+0.35</td>
<td>+0.47</td>
<td>-0.18</td>
</tr>
<tr>
<td>PPi</td>
<td>µmole</td>
<td>+0.51</td>
<td>+0.33</td>
<td>+0.41</td>
<td></td>
</tr>
</tbody>
</table>

washed three times with 0.3 ml of 0.1 n HCl, and the combined supernatant and washings were assayed for phosphate. The washing procedure is essential for the recovery of adsorbed orthophosphate.

Because of the relatively high level of PPi in Experiment 4, a satisfactory estimation of hydroxymethylpyrimidine-PP based on labile phosphate was not achieved. However, by adsorbing thiamine-P and thiazole-P on a cation exchange resin, hydroxymethylpyrimidine-PP could be determined spectrophotometrically. The reaction mixture was deproteinized by the addition of 0.1 ml of 2.5 M perchloric acid, 0.75 ml of the filtrate was collected. The absorption spectrum of the column eluate was calculated from the extinction at 246 nm corrected for the extinction of similarly treated zero time controls. This correction, due to nonspecific absorption contributed by the resin, was less than 5%.

Effect of Substrates and Inhibition by Pyrophosphate—Kinetic studies at very low levels of substrate were facilitated by the high sensitivity of the CNBr fluorimetric method for thiamine-P. The concentrations of hydroxymethylpyrimidine-PP and of thiazole-P decreased by less than 5% during the 5-minute incubation. Figs. 3 and 4 show the effect of each substrate and of 8 X 10^{-5} µmole PPi on the rate of synthesis of thiamine-P. The inhibition by PPi is not competitive with either substrate. In similar experiments, increasing the Mg++ concentration to approximately 5 times the concentration required to saturate the enzyme, did not overcome the inhibition by PPi. The Michaelis constants, estimated from double reciprocal plots (25), were 1 X 10^{-5} M for hydroxymethylpyrimidine-PP and 7 X 10^{-5} M for thiazole-P.

Equilibrium—The equilibrium of the reaction at pH 0.2 was studied in both directions by measuring the change in thiamine-P concentration under conditions favoring synthesis or pyrophosphorolysis (Fig. 5). The addition of inorganic pyrophosphatase after equilibrium had been reached in the forward direction, caused the reaction to go to completion and indicated that the enzyme was still active. The reverse reaction was studied in the presence of a 5-fold excess of PPi. An equilibrium constant for Reaction 4 as written, was determined for eight such experiments; the values ranged from approximately 4.5 to 9 with an average of 6. In favoring synthesis, this reaction resembles the formation of nicotinic acid mononucleotide and nicotinamide mononucleotide by similar pyrophosphate displacement reactions (26, 27). In contrast, the synthesis of nicotinamide riboside from ribose-1-phosphate and nicotinamide by phosphate displacement strongly favors phosphorolysis (28).

Enzymatic Assay of Hydroxymethylpyrimidine-PP and Thiazole-P—In the presence of an excess of one substrate, the limiting component can be quantitatively converted to thiamine-P by using an enzyme preparation containing inorganic pyrophosphatase. The thiamine-P is determined fluorimetrically in the

![Fig. 3](http://www.jbc.org/)

**Fig. 3.** The rate of thiamine-P formation as a function of hydroxymethylpyrimidine-PP (PYPP) concentration in the presence (○) and absence (●) of 8 X 10^{-5} M PPi. The reaction mixture (0.5 ml) contained, in addition to hydroxymethylpyrimidine-PP and PPi, thiazole-P, 0.05 µmole; glycine buffer, pH 9.2, 50 µmoles; mercaptoethanol, 0.5 µmole; MgSO4, 0.5 µmole; and approximately 2 X 10^{-4} units of enzyme. After 5 minutes at room temperature, the reaction was stopped by the addition of 0.025 ml of 2.5 n HCl and assayed for thiamine-P by the CNBr procedure.

![Fig. 4](http://www.jbc.org/)

**Fig. 4.** The rate of thiamine-P formation as a function of thiazole-P concentration in the presence (○) and absence (●) of 8 X 10^{-5} M PPi. The reaction conditions were the same as for Fig. 3 except that the reaction mixture contained approximately 3 X 10^{-4} units of enzyme and 0.1 µmole of hydroxymethylpyrimidine-PP.
Fig. 5. Reversibility and equilibrium. The reaction mixture (1.0 ml) contained MgSO$_4$, 0.1 μmole; mercaptoethanol, 0.5 μmole; glycine buffer, pH 9.2, 100 μmoles; and approximately 0.1 unit of enzyme. In pyrophosphorolysis studies, • and △ contained, respectively, 0.074 and 0.044 μmole of thiamine-P and 0.46 and 0.26 μmole of hydroxymethylpyrimidine-PP. At the arrows, 5 units (4) of highly purified inorganic pyrophosphatase were added. Aliquots were hydroxymethylpyrimidine-PP. At the arrows, 5 units (4) of highly purified inorganic pyrophosphatase were added. Aliquots were diluted with 0.125 M NaOH to a volume of 1.0 ml. The conditions are the same as for Fig. 5 except that the incubation mixture contained 0.1 to 0.5 unit of enzyme and approximately 2 mμmole of one substrate and 5 mμmole of the other. The reaction goes to completion in less than 20 minutes at 37°C. Analytical results agree within 5% with those based on phosphate analysis of each substrate.

DISCUSSION

The results of this study show that the enzymatic synthesis of thiamine requires the participation of phosphorylated forms of its two cyclic constituents; the thiazole as a monophosphate and the pyrimidine as a pyrophosphate (Fig. 6). The phosphorylation of the alcohol group of the thiazole provides a possible binding group and an element of structural specificity, but is not otherwise noteworthy. Although the pyrophosphorylation of the hydroxyl group of the pyrimidine may involve these same factors, a more significant aspect of the resulting compound is that the pyrophosphorylated carbon is allylic in a manner analogous to a benzyl derivative. Allylic compounds (29, 30) are highly reactive in nucleophilic substitution reactions, because both the removal of the leaving group and the bonding to the nucleophilic reagent are facilitated by resonance. In hydroxymethylpyrimidine-PP, the adjacent amino group would contribute to the resonance of the allylic structure. The relative acid lability of the pyrimidine phosphate groups, in contrast to the stability of the phosphate group of thiazole phosphate and of thiamine phosphate is an indication of this activation.

Other examples of allylic activation indicate that this mechanism may be of more general significance in biosynthetic reactions. It has been demonstrated (31, 32) that the key isoprenoid unit involved in the synthesis of squalene, rubber, and higher terpenes is γ,γ-dimethylallyl pyrophosphate. A similar activation of the hydroxymethyl group of the pteridine, 2-amino-4-hydroxy-6-hydroxymethyl dipyridyldisulfane in the biosynthesis of folic acid is suggested by the work of Jaenicke and Chan (33) and Shiotia and Disraley (34).

The enzymatic synthesis of thiamine phosphate, viewed as a reversible base exchange with the pyrophosphate anion as a conjugate base, is analogous to the sulfite cleavage of thiamine and to the action of thiaminase. The observation (35) that sulfite catalyzes a similar reaction between thiamine and various primary and tertiary amines suggests that the pyrimidyl sulfonate or a related structure may serve as an intermediate in a manner analogous to hydroxymethylpyrimidine-PP. Both thiaminase and thiamine-P pyrophosphorylase may act by forming a pyrimidyl-enzyme complex analogous to the adenosine diphosphate ribose-enzyme intermediate proposed (2) for the mechanism of DPNase. Studies with P$_32$-labeled pyrophosphate may show that such an intermediate is formed in the mechanism of thiamine-P pyrophosphorylase.

The experiments of Shimazono et al. (36) have provided elegant evidence in support of the conclusion (37, 38) that thiamine-P is not an intermediate in conversion of thiamine to thiamine-PP. Thus, thiamine-P must first be dephosphorylated before it can be converted to its biologically useful form by transpyrophosphorylation from ATP. This uncoupling in the synthesis of thiamine-PP, by the interposition of two essentially irreversible reactions, may be of some advantage in maintaining the cellular level of the coenzyme by isolating it from the reversible reaction leading to its synthesis.

SUMMARY

1. An enzyme that catalyzes the synthesis of thiamine phosphate from its constituent pyrimidine and thiazole moieties has been purified approximately 500-fold from baker's yeast, and some of its properties have been studied.

2. The enzyme requires Mg$^{++}$ for optimal activity and is specific for the pyrophosphate of the pyrimidine and the monophosphate of the thiazole. The reaction is freely reversible and has an apparent equilibrium constant of approximately 6 at the pH optimum, 9.2. The relationship of this reaction to the cleavage of thiamine by sulfite and by the enzyme thiaminase has been discussed.

3. The synthesis of the pyrimidyl monophosphate and pyrophosphate has been described. The allylic structure of these compounds and the significance of allylic activation in biosynthetic reactions have been discussed.

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The Enzymatic Synthesis of Thiamine Monophosphate
Irwin G. Leder


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